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2-Aminopurine Derivatives with C6-Substituted Olefin as Novel Cross-Linking Agents and the Synthesis of the Corresponding β-Phosphoramidite Precursors

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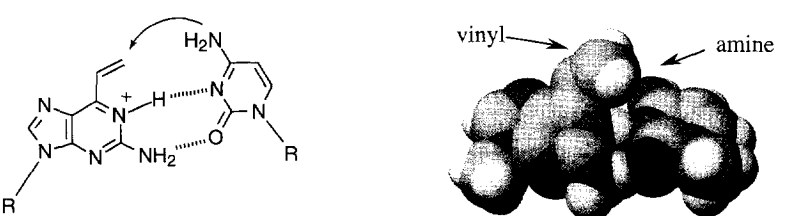
Abstract The 6-vinylated 2-aminopurine nucleoside (**1**), which was prepared by the Pd(0)-catalyzed cross-coupling reaction using guanosine 6-*O*-tosylate and vinyltributylstannane, has been demonstrated as a potential cross-linking agent. However, attempts for its incorporation into oligonucleotides were unsuccessful because of the high reactivity toward nucleophiles. In this study, new 2'-deoxy nucleoside derivatives with 6-(2-substituted vinyl)-2-aminopurine were designed to diminish the reactivity of the vinyl group. These new nucleosides have been shown to maintain reactivity toward potent nucleophiles such as butylamine and thiols, suggesting that they would form cross-linking with the target nucleobase due to the proximity within the sense-antisense duplex. Thus, the corresponding β-phosphoramidite precursors were successfully prepared, and were applied to an automated oligonucletotide synthesizer.

Oligodeoxynucleotides (ODNs) have been used as powerful tools for specific inhibition of translation of the *m*RNA in the antisense strategy,[1] and the targets for their application have been recently expanded to the duplex DNA by triplex formation in the so-called antigene strategy.[2] These applications *in vivo* have attracted great attention for therapeutic use. The efficacies of inhibition by the ODNs rely on the following: 1) the ability to bind with its complementary targets, 2) stability of the complementary complexes, and 3) resistance of the ODNs toward *in vivo* metabolism. Cross-linking has been a potential method to stabilize sense-antisense complexation,[3-5] and has been widely applied to *in vitro* experiments.[6] However, its *in vivo* use has been limited because of chemical instability[7] or slow reactivity[8] of the cross-linking agents. We recently designed the 6-vinyl-2-aminopurine analog (**1**) and demonstrated its potential as a new cross-linking agent.[9]



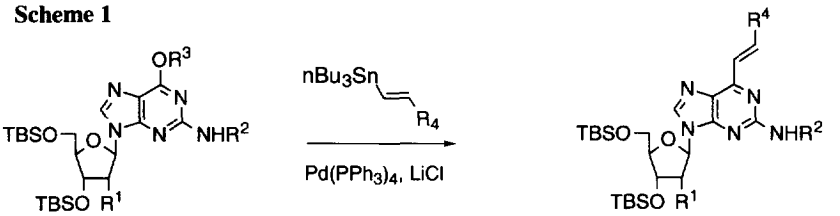
1

Fig. 1. Expected Complex Structure Between Protonated **1** and Cytosine. The space filling model (the right, R=Me) was generated by MOPAC (PM3) calculation and indicates the 6-vinyl of **1** and the amino group of cytosine in proximity.

During further investigation for evaluation of its cross-linking ability using oligonucleotides, it turned out that derivatization of **1** for ODN synthesis was not practical because of the high reactivity of its vinyl group toward a variety of nucleophiles. Thus, additional methyl- or trimethylsilyl substitution at the vinyl group was designed to diminish the reactivity of **1**.[10] In this paper, we describe in detail that these modifications have enabled the synthesis of the corresponding β-phosphoramidite precursors which are applicable to an automated DNA synthesizer.

6-Vinyl-2-aminopurine (**1**) was originally designed based on the following expectations: 1) the conjugated vinyl group may act as an alkylating group, 2) reactivity and stability of the vinyl group may be adjustable with an additional functional group, 3) cross-linking is expected to occur at a base pair with cytosine (Fig. 1). The protonated form of **1** was supposed to form a complex with cytosine to bring the vinyl and the amino groups into proximity; this assumption was supported by MOPAC (PM3) calculation (Fig. 1).

Scheme 1



2~5 6~14

Table 1. Cross-Coupling Reaction with $Pd(PPh_3)_4$ as the Catalyst.

Entry	Compd	R^1	R^2	R^3	R^4	Condition (h)[a]	Yield (%)[b]	Product
1	**2**	OTBS	Ac	Tf	H	B (24)	45	**6**
2	**2**	OTBS	Ac	Tf	H	A (0.75)	74	**6**
3	**3**	OTBS	H	Ts	H	A (3)	82	**7**
4	**3**	OTBS	H	Ts	TMS	A (3)	94	**8**
5	**4**	H	H	Ts	H	A (1)	94	**9**
6	**4**	H	H	Ts	Me[c]	A (3)	86	**10**[c]
7	**4**	H	H	Ts	TMS	A (3)	quant.	**11**
8	**5**	H	CO*i*Pr	Ts	H	A (2)	65	**12**
9	**5**	H	CO*i*Pr	Ts	Me[c]	A (2)	90	**13**[c]
10	**5**	H	CO*i*Pr	Ts	TMS	A (2)	63	**14**

a) The mixture was heated at 100 °C in dioxane (A) or at 60 °C in toluene (B). b) Isolated yield. c) Mixture of E- and Z-isomers.

6-Carbon substituted purines have been synthesized *via* nucleophilic substitution of 6-*O*-tosylates of guanosine derivatives by active methylene compounds[11] as well as palladium-catalyzed cross-coupling reactions using 6-chloropurines.[12] We also found that a 6-*O*-triflate derivative of guanosine afforded 6-vinylated compounds through a Pd(0)-catalyzed reaction with vinyltributylstannane in the presence of LiCl (Scheme 1).[13] The cross-coupling did not take place when $PdCl_2(PPh_3)_2$ was used as the catalyst. It should be noted that 6-*O*-tosylates also produced 6-vinylated derivatives in higher yields under the same conditions (Table 1).[9] As the 6-*O*-tosylates can be prepared easily and are very stable, they appear to be superior substrates in this cross-coupling reaction. All the 6-vinylated 2-aminopurines used in this study were synthesized in high yields by this new cross-coupling reaction (Table 1).

The reactivity of the terminal olefin of **7** toward nucleophiles was investigated by following the adducts formation (Scheme 2). It was found that the addition occurs in the presence of an acid catalyst such as *p*-toluenesulfonic acid or camphor-10-sulfonic acid (Table 2). The adduct was formed rapidly with the thiol nu-

cleophile in the absence of the acid catalyst. The reactivity is in the order of thiol>amine>alcohol, the same as the order of nucleophilicity (entries 1-3). Among four nucleosides, cytidine and guanosine derivatives formed adducts with **7**. The adduct structures with cytidine (Fig. 2, A) and guanosine (Fig. 2, B) were determined by 1D, 2D ^{1}H-NMR, MS/MS FAB and high resolution FAB mass spectra. The alkylation of N-4 of cytidine in the adduct A was evidenced by ^{1}H-NMR spectrum, in which the signal of the C-6 proton showed high-field shift to 7.45 ppm compared to 8.23 ppm in cytidine. Also, the fact that the signal of the C-8 proton shifted to down-field from 7.91 ppm in guanosine to 9.15 ppm in the adducts B indicates that **7** alkylated guanosine at N-7.

Scheme 2

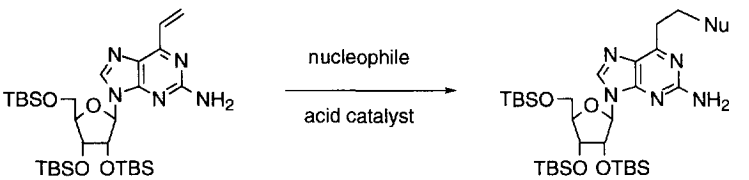


Table 2. Adduct Formation Between **7** and Several Nucleophiles.

Entry	Nucleophile	Time (h)	Adduct (%)[a]	Recovered **7** (%)[a]
1[b]	L-cystein	1	**15**: 96	0
2	aniline	1	**16**: 90	0
3[c]	MeOH	30	**17**: 44	0
4[d]	cytidine[e]	4	**18**: 17 (30)	41 (70)
5[d]	guanosine[e]	4	**19**: 27(35)	10 (65)
6	thymidine[e]	24	no adduct	quantitative
7	adenosine[e]	24	no adduct	decomposition

a) Isolated yields. Data in parenthesis indicate the ratios obtained by ^{1}H-NMR. b) The reaction was done using a solution of L-cystein methyl ester hydrochloride in ethanol in the absence of CSA. c) The reaction was done in methanol in the presence of CSA. d) Repetitive purification lowered the isolated yields. e) Protected nucleosides with TBDMS.

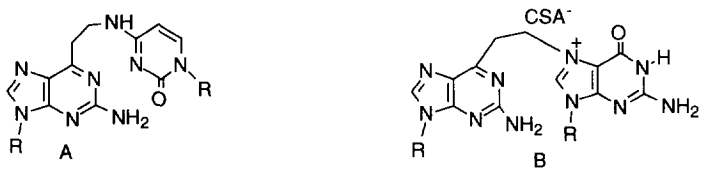


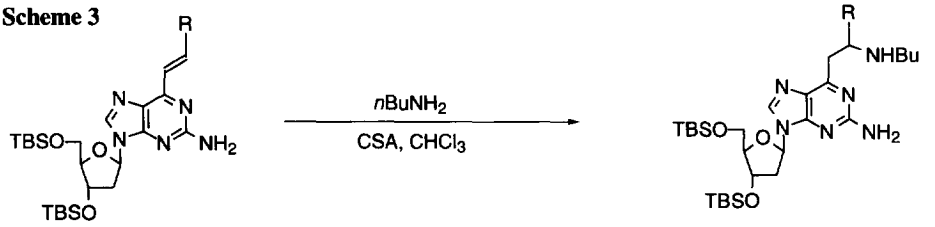
Fig. 2. The Adducts between **7** and Cytidine (A) or Guanosine (B). (R=tri TBDMS protected ribosyl).

The reaction with these nucleosides seemed to attain equilibrium, since the adduct-to-**7** ratios did not change after 4 hours, and the adducts isolated were decomposed to a mixture of each component in solution in the presence of CSA. From these adduct structures (Fig. 2), it was supposed that **1** in the ODN would cross-link with cytidine within sense-antisense hybrids. In addition, since N-7 of a guanosine appears within the major groove of the DNA duplex, **1** would form cross-link with a guanosine when the ODN incorporating **1** is applied to the triplex formation.[14]

Subsequently, a 2'-deoxy derivative of **7** was prepared by a similar procedure as described above, and its incorporation into ODNs was investigated. However, the 6-vinyl group was found to suffer from nucleophilic attack with a variety of reagents, such as those used in the phospholylation. We then attempted to diminish the

reactivity of the vinyl group with an additional substituent. Prior to the experiment, activation energies of the reaction between 6-(2-substituted vinyl)-2-aminopurine derivatives and ammonia were estimated by a semi-empirical MO calculation (MOPAC (PM3)),[15] and it was suggested that reactivity of vinyl derivatives might be lowered in the order of R= H>methyl>silyl ($\Delta G^{\neq}$ in Table 3). Thus, 2'-deoxyribosyl-6-(2-substituted vinyl)-2-aminopurine derivatives (**9**, **10**, **11**) were synthesized *via* Pd(0)-catalyzed cross-coupling, and their reactivity toward *n*-butylamine was compared (Scheme 3). It was revealed that the rate for the adduct formation is in the order of **9>10>11** as expected based on the MO calculation. The tendency of the protonation to N-1 of the purine skeleton may not correlate to the reactivity, because these compounds have similar p*Ka* values (**9**: 3.7, **10**: 4.0, **11**: 3.9).[16] Neither **10** nor **11** formed adducts with cytidine or guanosine, but the substituted vinyl groups apparently maintain reactivity toward nucleophiles. Therefore, they may be expected to form cross-linking with cytidine due to the proximity effect within the sense-antisense duplex when these compounds are incorporated into ODNs.

Scheme 3



9: R=H
10: R=Me
11: R=TMS

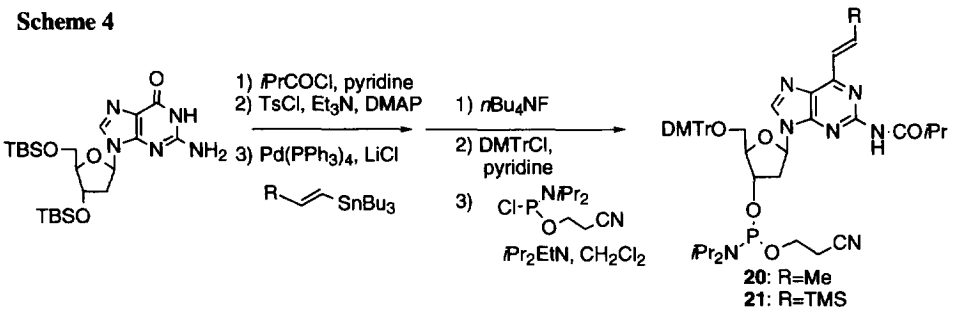
Table 3.

Compd	R	$t_{1/2}$ (h)[a]	$\Delta G^{\neq}$(kcal/mol)[b]
9	H	1.5	11.2
10	Me	10	12.1
11	TMS	>48	13.7

a)Experimental results. b) Calculated values by MOPAC.

During the synthesis of β-phosphoramidite derivatives and the application to the ODN synthesis, we noticed that the 2-amino group reacted with the β-phosphoramidite derivative. Accordingly, 2-NH_2 of 2'-deoxy guanosine was protected with an isobutyryl group prior to the vinylation, then converted to the β-phosphoramidite derivatives by a sequence of reactions including deprotection with nBu$_4$NF, protection with DMTrCl, and reaction with 2-cyanoethyl *N,N*-diisopropylchlorophosphoamidite (Scheme 4).[17] These β-phosphoramidite derivatives were successfully applied to an automated DNA synthesizer to produce oligonucleotides incorporating **10** or **11**.[18]

Scheme 4



In conclusion, we synthesized 6-(2-substituted vinyl)-2-aminopurine derivatives, and successfully demonstrated its potential as a new cross-linking agent by achieving adduct formation with cytidine and guanosine

in the presence of an acid catalyst. Acceleration by the acid catalyst may be beneficial, since the grooves of DNA are thought to be in an acidic environment.[19] The new 6-(2-substituted vinyl)-2-isobutyrylaminopurine analogs with the substituted-vinyl group have been developed to decrease the reactivity to avoid the side reactions observed in the incorporation into the ODNs.[18] The corresponding β-phosphoramidite precursors were successfully synthesized and applied to an automated DNA synthesizer. Further study is ongoing for the synthesis of oligonucleotides incorporating the new 2-aminopurine nucleosides, and their application to sequence specific cross-linking.

Experimental

Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR Spectra were taken in $CDCl_3$ or CD_3OD either at 500 MHz or at 270 MHz. Analytical TLC was carried out on Merck precoated TLC plates (Kieselgel 60 F_{254}, 0.2 mm). Column chromatography was done by using FL60D or BW-200 (Fuji Silysia).

N^2-Acetyl-2',3',5'-tri-*O*-*tert*-butyldimethylsilyl-O^6-trifluoromethanesulfonylguanosine (2). A suspension of guanosine (2.0 g, 7.0 mmol), *t*-butylchlorodimethylsilane (4.8 g, 32 mmol) and imidazole (4.8g, 70 mmol) in dry dimethylformamide (DMF, 40 mL) was stirred for 23 h at room temperature, then diluted with ethyl acetate (100 mL). The separated organic layer was washed successively with H_2O and brine, then dried over Na_2SO_4. Evaporation of the solvent gave a crude oil, which was chromatographed on a silica gel column (chloroform:methanol=95:5) to give 2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine as a colorless syrup (4.4 g, quant.). IR (cm^{-1}, neat): 3300, 3100, 1690; ^{1}H-NMR ($CDCl_3$) δ:12.03 (1H, s), 7.91 (1H, s), 6.28 (2H, bs), 5.83 (1H, d, 4.3 Hz), 4.43 (1H, t, *J*=4.3 Hz), 4.28 (1H, t, *J*=4.6 Hz), 4.12-4.08 (1H, m), 3.99 (1H, dd, *J*=11.5, 3.6 Hz), 3.79 (1H, dd, *J*=11.5, 2.3 Hz), 0.94 (9H, s), 0.93 (9H, s), 0.87 (9H, s), 0.14 (3H, s), 0.13 (3H, s), 0.10 (3H, s), 0.09 (3H, s), 0.02 (3H, s), 0.00 (3H, s); FDMS (m/z): 626 $(M)^+$.

Acetic anhydride (0.18 mL, 1.9 mmol) was added to a solution of the above *O*-protected guanosine (230 mg, 0.37 mmol) in dry pyridine (2 mL) under argon. The reaction mixture was stirred at 110 ℃ for 9 h, and cooled to room temperature, then quenched with methanol for 10 min. The mixture was diluted with chloroform and washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and evaporated to give the crude product, which was chromatographed on a silica gel column (chloroform:methanol=10:0 to 20:1) to give N^2-acetyl-2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine as an orange syrup (234 mg, 95%). IR (cm^{-1}, neat): 3150, 3050, 1680, 1600; ^{1}H-NMR ($CDCl_3$) δ:11.89 (1H, brs), 8.35 (1H, brs), 8.06 (1H, s), 5.86 (1H, d, *J*=5.6 Hz), 4.35 (1H, dd, *J*=5.3, 4.3 Hz), 4.25 (1H, dd, *J*=4.3, 3.0 Hz), 4.09 (1H, q, *J*=2.6 Hz), 3.91 (1H, dd, *J*=11.6, 3.0 Hz), 3.77 (2H, dd, *J*=11.6, 2.3 Hz), 2.28 (3H, s), 0.95 (9H, s), 0.94 (9H, s), 0.79 (9H, s), 0.14 (3H, s), 0.13 (3H, s), 0.11 (3H, s), 0.10 (3H, s), -0.04 (3H, s), -0.25 (3H, s); FDMS (m/z): 668 $(M)^+$.

Trifluoromethanesulfonyl chloride (0.28 mL, 2.0 mmol) was added into a solution of triethylamine (0.28 mL, 2.0 mmol) and the above product (433 mg, 0.65 mmol) in dichloromethane (3 mL) under argon at -40 °C , and the mixture was stirred at the same temperature for 90 min, followed by the addition of triethylamine (0.28 mL) and trifluoromethanesulfonyl chloride (0.21 mL). The reaction mixture was stored at -30 °C for 19 h, and diluted with ethyl acetate, then washed successively with H_2O and brine. The organic layer was dried (Na_2SO_4) and evaporated to give the crude product, which was chromatographed on a silica gel column (hexane:ethyl acetate=10:1 to 1:1) to give **2** as a pale yellow syrup (275 mg, 52%) and the recovered N^2-acetyl-2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine (100 mg, 23%). IR (cm^{-1}, neat): 3250, 1680; ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, s), 8.14 (1H, brs), 6.05 (1H, d, *J*=5.6 Hz), 4.62-3.74 (5H, m), 2.58 (3H, s), 0.95 (9H, s), 0.93 (9H, s), 0.72 (9H, s), 0.14 (6H, s), 0.11 (6H, s), -0.05 (3H, s), -0.30 (3H, s); FDMS (m/z): 800 $(M+1)^+$.

2',3',5'-Tri-*O*-*tert*-butyldimethylsilyl-O^6-*p*-toluenesulfonylguanosine (3). *p*-Toluenesulfonyl chloride (*p*-TsCl, 210 m g, 1.1 mmol) was added into a solution of triethylamine (0.15 mL, 1.1 mmol), dimethylaminopyridine (DMAP, 14 mg, 0.11 mmol) and 2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine (350 mg, 0.56 mmol) in dichloromethane (10 mL) at 0 °C , and the mixture was stirred for 12 h under argon at room temperature, then diluted with dichloromethane. The mixture was washed successively with H_2O and brine. The organic layer was dried (Na_2SO_4) and evaporated to give the crude product, which was chromatographed on a silica gel column (chloroform:methanol=100:0 to 98:2) to give **3** (330 mg, 75%) as a colorless oil. IR (cm^{-1}, neat): 3300, 1610; ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, s), 8.03 (2H, d, *J*=8.3 Hz), 7.35 (2H, d, *J*=7.9 Hz), 5.90 (1H, d, *J*=5.0 Hz), 4.98 (2H, brs), 4.45 (1H, t, *J*=4.6 Hz), 4.26 (1H, t, *J*=4.0 Hz), 4.09 (1H, q, *J*=3.6 Hz), 3.96 (1H, dd, *J*=11.5, 3.6 Hz), 3.76 (1H, dd, *J*=11.5, 2.6 Hz), 2.45 (3H, s), 0.94 (9H, s), 0.92 (9H, s), 0.81 (9H, s), 0.12 (3H, s), 0.10 (3H, s), 0.09 (3H, s), 0.00 (3H, s), -0.03 (3H, s), -0.20 (3H, s); FDMS (m/z): 780 $(M+1)^+$.

3',5'-Di-*O*-*tert*-butyldimethylsilyl-O^6-*p*-toluenesulfonyl-2'-deoxyguanosine (4). 2'-Deoxyguanosine (1.9 g, 6.7 mmol) was silylated as described above to give 3',5'-di-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine (2.8 g, 85%) as a colorless powder. mp 294-295 °C (decomp.); IR (cm^{-1}, nujol): 3200, 1680; ^{1}H-NMR ($CDCl_3$) δ: 12.03 (1H, s), 7.84 (1H, s), 6.24 (3H, m), 4.60-4.55 (1H, m), 3.97 (1H, dd, *J*=7.3, 3.5 Hz), 3.81 (1H, dd, *J*=11.6, 4.6 Hz), 3.75 (1H, dd, *J*=11.6, 3.8 Hz), 2.97-2.56 (1H, m), 2.36 (1H, ddd, *J*=13.2, 6.3, 4.2 Hz), 0.91 (18H, s), 0.11 (6H, s), 0.084 (3H, s), 0.080 (3H, s); FDMS (m/z): 601 $(M)^+$; Anal. Calcd for $C_{22}H_{41}N_5O_4Si_2$: C, 52.77; H, 8.17; N, 13.92. Found: C, 53.33; H, 8.28; N, 14.14.

The above product (1.1 g, 2.3 mmol) was tosylated as described above to give **4** (1.4 g, 96%) as a colorless powder. mp 171-172 °C ; IR (cm^{-1}, nujol): 3500, 3300, 3180, 1680; ^{1}H-NMR ($CDCl_3$) δ: 8.02 (1H, s), 8.03 (2H, d, *J*=8.6 Hz), 7.36 (2H, d, *J*=7.9 Hz), 6.30 (1H, t, *J*=6.6 Hz), 5.05 (2H, brs), 4.57 (1H, m), 3.98 (1H, dd, *J*=6.9, 3.3 Hz), 3.81 (1H, dd, *J*=11.2, 4.3 Hz), 3.75 (1H, dd, *J*=11.2, 3.0 Hz), 2.58-2.49 (1H, m), 2.45 (3H, s). 2.36 (1H, ddd, *J*=13.2, 5.9, 3.6 Hz). 0.91 (9H, s), 0.90 (9H, s), 0.10 (6H, s), 0.08 (3H, s), 0.07 (3H, s); FDMS (m/z): 650 $(M+1)^+$; Anal. Calcd for $C_{29}H_{47}N_5O_6SSi_2$: C, 53.69; H, 7.21; N, 10.64; Found: C, 53.62; H, 7.24; N, 10.79.

N^2-Isobutyryl-3',5'-Di-*tert*-butyldimethylsilyl-O^6-*p*-toluenesulfonyl-2'-deoxyguanosine (5). Isobutyryl chloride (0.09 mL, 0.84 mmol) was added into a solution of 3',5'-di-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine (200 mg, 0.4 mmol) in pyridine (5 mL) at 0 °C under argon. The mixture was stirred for 3 h and diluted with dichloromethane, then washed successively with 10% aqueous NaOH, 10% aqueous HCl and brine. The organic layer was dried (Na_2SO_4) and evaporated to give the residue, which was recrystallized from MeOH to afford N^2-isobutyryl-3',5'-di-*O*-*tert*-butyldimethylsily-2'-deoxyguanosine (210 mg, 92%) as a colorless powder. mp 114 °C; IR (cm^{-1}, nujol): 3300, 1700;1680; ^{1}H-NMR ($CDCl_3$) δ: 12.0 (1H, bs), 8.30 (1H, bs). 8.00 (1H, s), 6.22 (1H, t, *J*=6.3 Hz), 4.57 (1H, dd, *J*=3.3, 2.0 Hz), 3.97 (1H, dd, *J*=6.9, 3.3 Hz), 3.76 (2H, dd, *J*=11.6, 3.3 Hz), 2.68 (1H, dt, *J*=13.9, 6.9 Hz), 2.46-2.35 (2H, m), 1.27 (3H, d, *J*=6.9 Hz), 1.26 (3H, d, *J*=6.9 Hz), 0.90 (9H, s), 0.89 (9H, s), 0.11 (6H, s), 0.09 (6H, s), 0.07 (6H, s); FABMS (m/z): 556 (M^++1); HRFABMS calcd for $C_{26}H_{48}O_5N_5Si_2$ 556.3194, found 556.3205.

The above product (1.4 g, 2.5 mmol) was tosylated as described above to give **5** (757 mg, 42%) as a colorless syrup. IR (cm^{-1}, neat): 3300, 1720, 1620; ^{1}H-NMR ($CDCl_3$) δ: 8.23 (1H, s), 8.06 (2H, d, *J*=8.6 Hz), 7.87 (1H, bs), 7.38 (2H, d, *J*=8.6 Hz), 6.39 (1H, t, *J*=6.6 Hz), 4.60 (1H, dt, *J*=5.6, 3.3 Hz), 4.00 (1H, dd, *J*=10.2, 3.3 Hz), 3.86 (1H, dd, *J*=11.2, 4.0 Hz), 3.76 (1H, dd, *J*=11.2, 3.3 Hz), 3.21-3.14 (1H, m), 2.60 (1H, dt, *J*=13.9, 6.9 Hz), 2.46 (3H, s), 2.46-2.35 (1H, m), 1.26 (6H, d, *J*=6.6 Hz), 0.91 (9H, s), 0.90 (9H, s), 0.10 (3H, s), 0.09 (3H, s), 0.08 (6H, s); FABMS (m/z): 720 $(M+1)^+$. HRFABMS calcd for $C_{33}H_{54}N_5O_7Si_2S$ 720.3283, found 720.3287.

N^2-Acetyl-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-6-vinylpurine (6). A solution of LiCl (20 mg, 0.46 mmol), $Pd(PPh_3)_4$ (53 mg, 0.046 mmol) and **2** (181 mg, 0.23 mmol) in dioxane (3 mL) was stirred under argon for 10 min at room temperature, followed by the addition of vinyltributylstannane (0.38 mL, 1.2 mmol), then the mixture was heated under reflux for 45 min. The reaction mixture was diluted with ethyl acetate and washed successively with 10% aqueous NH_3 and brine. The organic layer was dried (Na_2SO_4) and evaporated to give the crude product, which was chromatographed on a silica gel column (hexane:ethyl acetate=19:1 to 9:1) to give **6** (114 mg, 74%) as a yellow syrup. IR (cm^{-1}, nujol): 3250, 3100, 1680; ^{1}H-NMR ($CDCl_3$) δ: 9.39 (1H, brs), 8.49 (1H, s), 7.17 (1H, dd, *J*=17.7, 11.0 Hz), 7.00 (1H, dd, *J*=17.7, 2.4 Hz), 6.06 (1H, d, *J*=6.7 Hz), 5.93 (1H, dd, *J*=11.0, 2.4 Hz), 5.27 (1H, brs), 4.45 (1H, dd, *J*=4.3, 2.4 Hz), 4.16 (1H, dd, *J*=11.0, 6.1 Hz), 4.14-4.10 (1H, m), 3.85 (1H, dd, *J*=11.0, 3.7 Hz), 2.39 (3H, s), 0.99 (9H, s), 0.95 (9H, s), 0.77 (9H, s), 0.21 (3H, s), 0.19 (3H, s), 0.16 (3H, s), 0.14 (3H, s), -0.005 (3H, s), -0.32 (3H, s); FABMS (m/z): 720 $(M+1)^+$, 620 (M-58).

2-Amino-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-6-vinylpurine (7). The tosylate **3** (395 mg, 0.51 mmol) was vinylated as described above to give **7** (267 mg, 82%) as a colorless oil. IR (cm^{-1}, neat): 3300, 1680, 1600; ^{1}H-NMR ($CDCl_3$) δ: 8.25 (1H, s), 7.17 (1H, dd, *J*=17.5, 10.6 Hz), 6.96 (1H, brd, *J*=17.8 Hz), 5.95 (1H, d, *J*=4.6 Hz), 5.91 (1H, d, *J*=11.9 Hz), 5.15 (2H, brs), 4.55 (1H, t, *J*=4.0 Hz), 4.29 (1H, t, *J*=4.6 Hz), 4.11 (1H, dd, *J*=6.3, 3.6 Hz), 3.98 (1H, dd, *J*=11.5, 4.0 Hz), 3.78 (1H, dd, *J*=11.6, 3.0 Hz), 0.96 (9H, s), 0.94 (9H, s), 0.82 (9H, s), 0.14 (3H, s), 0.13 (3H, s), 0.11 (3H, s), 0.10 (3H, s), -0.02 (3H, s), -0.19 (3H, s); ^{13}C-NMR($CDCl_3$) δ: 159.262 (s), 154.011 (s), 153.915 (s), 140.799 (d), 131.672 (d), 126.286 (s), 125.551 (t), 87.8011 (d), 85.231 (d), 75.708 (d), 71.950 (d), 62.574 (d), 26.121 (q), 25.877 (q), 25.717 (q), 18.569 (s), 18.532 (s), 18.116 (s), -4.345 (q), -4.590 (q), -4.688 (q), -4.945 (q), -5.312 (q), -5.349 (q); FABMS (m/z): 636 $(M)^+$, 578 $(M-58)^+$; Anal. Calcd for $C_{30}H_{57}N_5O_4Si_3 \cdot 0.5H_2O$: C, 55.85; H, 9.06; N, 10.86. Found: C, 55.59; H, 8.77; N, 10.71.

2-Amino-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-6-(2-trimethylsilylvinyl)purine (8). The tosylate **3** was vinylated as described above using trimethylsilyl vinyltributylstannane (2.5 g, 6.9 mmol) to give **8** (415 mg, 94%) as a colorless oil. IR (cm^{-1}, neat): 3300, 1680, 1600; ^{1}H-NMR ($CDCl_3$) δ: 8.29 (1H, s), 7.95 (1H, s), 7.77 (1H, d, *J*=18.8 Hz), 7.49 (1H, d, *J*=18.8 Hz), 6.08 (1H, d, *J*=6.3 Hz), 4.55 (1H, dd, *J*=5.9, 4.6 Hz), 4.26 (1H, dd, *J*=4.3, 2.6 Hz), 4.12 (1H, dd, *J*=5.9, 2.6 Hz), 3.96 (1H, dd, *J*=11.2, 3.6 Hz), 3.80 (1H, dd, *J*=11.2, 2.3 Hz), 2.64 (3H, s), 0.97 (9H, s), 0.95 (9H, s), 0.75

(9H, s), 0.23 (9H, s), 0.16 (3H, s), 0.14 (3H, s), 0.11 (6H, s), -0.06 (3H, s), -0.38 (3H, s); FABMS (m/z): 708 $(M+1)^+$; HRFABMS calcd for $C_{33}H_{66}N_5O_4Si_4$ 708.4158, found 708.4175.

2-Amino-9-(3,5-di-*O-tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)-6-vinylpurine (9). The tosylate **4** was vinylated as described above using vinyltributylstannane (2.5 g, 6.9 mmol) to give **9** (370 mg, 94%) as a colorless oil. IR (cm^{-1}, neat): 3500, 3300, 3200, 1620; ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, s), 7.15 (1H, dd, *J*=17.5, 10.9 Hz), 6.88 (1H, dd, *J*=17.5, 2.0 Hz), 6.36 (1H, t, *J*=6.6 Hz), 5.86 (1H, dd, *J*=10.9, 2.0 Hz), 5.03 (2H, brs), 4.62-4.58 (1H, m), 4.00 (1H, dd, *J*=4.7, 3.3 Hz), 3.82 (1H, dd, *J*=11.2, 4.3 Hz), 3.76 (1H, dd, *J*=11.2, 3.6 Hz), 2.61 (1H, ddd, *J*=13.2, 7.9, 5.3 Hz), 2.37 (1H, ddd, *J*=13.2, 6.1, 3.6 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.11 (6H, s), 0.083 (3H, s), 0.077 (3H, s); FABMS (m/z): 506 $(M+1)^+$, 1011$(2M+1)^+$; HRFABMS calcd for $C_{24}H_{44}N_5O_3Si_2$ 506.2989, found: 506.2986.

2-Amino-9-(2-deoxy-β-D-ribofuranosyl)-6-vinylpurine. A solution of **9** (36 mg, 69 μmol) and *n*Bu_4NF (1M THF solution, 0.2 mL, 210 μmol) in tetrahydrofuran (THF, 0.1 mL) was stirred under argon at room temperature for 1 h, and the mixture was directly chromatographed on a silica gel column (chloroform: methanol=95:5 to 9:1) to give the title compound (13 mg, 68%) as a colorless powder. mp 82-84 °C; IR (cm^{-1}, neat): 3100, 1650; ^{1}H-NMR (CD_3OD) δ: 8.26 (1H, s), 7.11 (1H, dd, *J*=17.5, 10.9 Hz), 6.77 (1H, dd, *J*=17.5, 2.0 Hz), 6.37 (1H, dd, *J*=7.6, 6.9 Hz), 5.83 (1H, dd, *J*=10.9, 2.0 Hz), 4.57 (1H, q, *J*=3.0 Hz), 4.03 (1H, dd, *J*=6.3, 3.6 Hz), 3.84 (1H, dd, *J*=12.2, 3.3 Hz), 3.74 (1H, dd, *J*=12.2, 4.0 Hz), 2.80 (1H, ddd, *J*=13.5, 7.6, 5.9 Hz), 2.38 (1H, ddd, *J*=13.5, 5.9, 3.0 Hz); FABMS (m/z): 278 $(M+1)^+$.

2-Amino-9-(3,5-di-*O-tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)-6-(1-propenyl)-purine (10). The tosylate **4** (619 mg, 0.95 mmol) was vinylated as described above using propenyltributyl-stannane (1.5 mL, 4.7 mmol, cis:trans=2.5:1) to give **10** (427 mg, 86%) as a colorless syrup (cis:trans=2:3). IR (cm^{-1}, neat): 3200, 1620; ^{1}H-NMR ($CDCl_3$) δ: 7.99 (1H, s), 7.44 (0.6H, dq, *J*=15.5, 6.9 Hz), 6.90 (0.4H, dq, *J*=11.9, 2.0 Hz). 6.85 (0.6H, dq, *J*=15.2, 1.7 Hz), 6.29 (0.4H, dq, *J*=11.6, 7.3 Hz), 6.36 (1H, dd, *J*=5.6, 1.9 Hz), 4.89 (2H, bd, *J*=4.0 Hz), 4.60 (1H, q, *J*=3.0 Hz), 3.99 (1H, q, *J*=3.3 Hz), 3.82 (1H, dd, *J*=11.2, 4.3 Hz), 3.75 (1H, dd, *J*=11.2, 3.6 Hz), 2.64-2.59 (1H, m), 2.40-2.31 (1H, m), 2.26 (1.2H, dd, *J*=7.3, 2.0 Hz), 2.02 (1.8H, dd, *J*=6.9, 1.7 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.11 (6H, s), 0.08 (3H, s), 0.07 (3H, s); FABMS (m/z): 520 $(M+1)^+$; HRFABMS calcd for $C_{25}H_{46}N_5O_3Si_2$ 520.3139, found 520.3143.

2-Amino-9-(2-deoxy-β-D-ribofuranosyl)-6-(1-propenyl)purine. The reaction of **10** (13 mg, 30 μmol) with *n*Bu_4NF (1M THF solution, 0.1 mL, 100 μmol) in THF (0.1 mL), and the subsequent purification as described above gave the title compound (7 mg, 98%) as a colorless powder. mp 74-76 °C ; IR (cm^{-1}, neat): 3500, 1650; ^{1}H-NMR (CD_3OD) δ: 7.77 (1H, s), 7.49 (0.6H, dq, *J*=15.5, 6.9 Hz), 6.89 (0.4H, dq, *J*=15.5, 2.0 Hz), 6.83 (0.6H, dq, *J*=15.5, 1.7 Hz), 6.34 (0.4H, dq, *J*=11.9, 7.3 Hz), 6.27 (1H, dd, *J*=9.2, 2.6 Hz), 5.01 (2H, b), 4.77 (1H, d, *J*=4.3 Hz), 4.22 (1H, s), 3.98 (1H, dd, *J*=12.5 Hz), 3.79 (1H, d, *J*=12.5 Hz), 3.18 (1H, m), 2.29 (1H, dd, *J*=13.5, 5.6 Hz), 2.26 (1.2 H, dd, *J*=7.3, 1.7 Hz), 2.03 (1.8 H, dd, *J*=6.9, 1.7 Hz); FABMS (m/z): 292 $(M+1)^+$; HRFABMS calcd for $C_{13}H_{18}N_5O_3$ 292.1410, found 292.1436; Anal. Calcd for $C_{13}H_{17}N_5O_3$•3/5H_2O: C, 51.68; H, 6.00; N, 23.19. Found: C, 51.95; H, 5.84; N, 22.84.

2-Amino-9-(3,5-di-*O-tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)-6-(2-trimethyl-silylvinyl)purine (11) The tosylate **4** (664 mg, 1.02 mmol) was vinylated as described above using trimethylsilylvinyltributylstannane (1.9 mL, 5.0 mmol) to give **11** (591 mg, 1.02 mmol, quant.) as a pale yellow syrup. IR (cm^{-1}, neat): 3500, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.02 (1H, s), 7.71 (1H, d, *J*=19.1 Hz), 7.37 (1H, d, *J*=19.1 Hz), 6.35 (1H, t, *J*=6.6 Hz), 4.94 (2H, bs), 4.59 (1H, m), 3.99 (1H, dd, *J*=10.9, 3.6 Hz), 3.81 (1H, dd, *J*=11.1, 4.5 Hz), 3.81 (1H, dd, *J*=11.2, 2.6 Hz), 2.66-2.56 (1H, m), 2.41-2.32 (1H, m), 0.91 (18H, s), 0.20 (9H, s), 0.08 (6H, s), 0.07 (6H, s); FABMS (m/z): 578 $(M+1)^+$; HRFABMS calcd for $C_{27}H_{52}N_5O_3Si_3$ 578.3378, found, 578.3393.

2-Amino-9-(2-deoxy-β-D-ribofuranosyl)-6-(2-trimethylsilylvinyl)purine. The reaction of **11** (8 mg, 10 μmol) with *n*Bu_4NF (1M THF solution, 0.03 mL, 30 μmol) in THF (0.1 mL) and the subsequent purification as described above gave the title compound (7 mg, 98%) as a colorless powder. mp 178 °C ; IR (cm^{-1}, neat): 3500, 3100, 1650; ^{1}H-NMR (CD_3OD) δ: 7.79 (1H, s), 7.75 (1H, dd, *J*=18.2 Hz), 7,34 (1H, d, *J*=18.5 Hz), 6.27 (1H, dd, *J*=9.5, 5.6 Hz), 5.01 (2H, b), 4.78 (1H, d, *J*=5.0 Hz), 3.99 (1H, q, *J*=6.9 Hz), 3.89 (1H, dd, *J*=12.9, 2.0 Hz), 3.79 (1H, bd, *J*=12.5 Hz), 3.14-3.05 (1H, m), 2.28 (1H, dd, *J*=13.5, 5.6 Hz), 0.21 (9H, s); FABMS (m/z): 350 $(M+1)^+$; Anal. Calcd for $C_{15}H_{23}N_5O_3Si$: C, 51.55; H, 6.63; N, 20.04. Found: C, 51.51; H, 6.67; N, 19.85.

2-Isobutyrylamino-9-(3,5-di-*O-tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)-6-vinylpurine (12). The tosylate **5** (500 mg, 0.69 mmol) was vinylated as described above using vinyl-tributylstannane (1.2 mL, 3.4 mmol) to give **12** (253 mg, 65%) as a pale yellow syrup. IR (cm^{-1}, neat):1680, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.25 (1H, s), 8.08 (1H, b), 7.23 (1H, dd, *J*=17.6, 10.6 Hz), 6.92 (1H, dd, *J*=17.8, 2.0 Hz), 6.44 (1H, t, *J*=6.6 Hz), 5.91 (1H, dd, *J*=10.8, 2.0 Hz), 4.63 (1H, dt, *J*=5.6, 3.3 Hz), 4.01 (1H, dd, *J*=3.3, 3.9 Hz), 3.86 (1H, dd, *J*=11.2, 4.0 Hz), 3.70 (1H, dd, *J*=11.2, 3.3 Hz), 3.23 (1H, b), 2.69-2.62 (1H, m), 2,47-2.41 (1H, m), 1.28 (6H, d, *J*=6.9 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.11 (6H, s), 0.08 (6H, s); FABMS (m/z): 576 $(M+1)^+$; HRFABMS calcd for $C_{28}H_{50}O_4N_5Si_2$ 576.3401, found 576.3405.

2-Isobutyrylamino-9-(3,5-di-*O-tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)-6-(1-propenyl)purine (13). The tosylate **5** (711 mg, 0.99 mmol) was vinylated as described above using

propenyltributylstannane (1.7 mL, 4.9 mmol) to give trans-**13** (340 mg, 58%) and cis-**13** (184 mg, 32%) as a pale yellow syrup. Physical data of trans-**13**: IR (cm^{-1}, neat): 3300, 1680, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.21 (1H, s), 7.91 (1H, s), 7.53 (1H, dq, *J*=15.8, 6.9 Hz), 6.94 (1H, dq, *J*=15.5, 1.7 Hz), 6.43 (1H, t, *J*=6.6 Hz), 4.62 (1H, dt, *J*=5.6, 3.3 Hz), 4.01 (1H, dd, *J*=3.3, 3.9 Hz), 3.86 (1H, dd, *J*=11.2, 4.0 Hz), 3.70 (1H, dd, *J*=11.2, 3.3 Hz), 3.23 (1H, b), 2.70-2.60 (1H, m), 2,46-2.41 (1H, m), 2.05 (3H, dd, *J*=6.9, 1.7 Hz), 1.28 (6H, d, *J*=6.9 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.11 (6H, s), 0.08 (6H, s); FABMS (m/z): 590 (M^++1), 532 (M^+-58); HRFABMS calcd for $C_{29}H_{52}O_4N_5Si_2$ 590.3558, found 590.3561.

2-Isobutyrylamino-9-(3,5-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)-6-(2-trimethylsilylvinyl)purine (14). The tosylate **5** (1.0 g, 1.39 mmol) was vinylated as described above using trimethylsilylvinyltributylstannane (2.7 mL, 6.9 mmol) to give **14** (575 mg, 63%) as a pale yellow syrup. IR (cm^{-1}, neat): 3300, 1650, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.25 (1H, s), 7.98 (1H, s), 7.80 (1H, d, *J*=19.1 Hz), 7.45 (1H, d, *J*=18.8 Hz), 6.44 (1H, t, *J*=6.6 Hz), 4.62 (1H, dt, *J*=5.6, 3.3 Hz), 4.01 (1H, dd, *J*=3.3, 3.9 Hz), 3.86 (1H, dd, *J*=11.2, 4.0 Hz), 3.70 (1H, dd, *J*=11.2, 3.3 Hz), 3.23 (1H, b), 2.70-2.60 (1H, m), 2,46-2.41 (1H, m), 1.29 (6H, d, *J*=6.9 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.22 (9H, s), 0.11 (6H, s), 0.08 (6H, s); FABMS (m/z): 648 (M^++1), 590 (M^+-58); HRFABMS calcd for $C_{31}H_{58}O_4N_5Si_3$ 648.3797, found 648.3790.

Adduct of 7 and L-cystein methyl ester (15). A solution of L-cystein methyl ester hydrochloride (6.0 mg, 28 μmol) and **7** (6.0 m g, 28 μmol) in dichloromethane-ethanol (0.1 mL-0.5 mL) was stirred at room temperature for 1 h, then the solvent was evaporated. The residue was chromatographed on a silica gel column (chloroform:methanol=99:1) to give **15** as a colorless oil (21 mg, 96%). ^{1}H-NMR ($CDCl_3$) δ: 8.08 (1 H, s), 5.86 (1H, d, *J*=4.6 Hz), 5.91-5.96 (2H, brs), 4.51 (1H, t, *J*=4.4 Hz), 4.24 (1H, d, *J*=4.0 Hz), 4.11 (1H, dd, *J*=6.1, 3.6 Hz), 3.98 (1H, dd, *J*=11.5, 3.6 Hz), 3.79 (1H, dd, *J*=11.6, 3.0 Hz), 3.78 (3H, s), 3.70-3.80 (2H, m), 3.51-3.70 (2H, m), 3.19-3.32 (2H, m), 3.00-3.16 (1H, m), 0.95 (9H, s), 0.92 (9H, s), 0.82 (9H, s), 0.14 (3H, s), 0.13 (3H, s), 0.11 (3H, s), 0.09 (3H, s), -0.012 (3H, s), -0.15 (3H, s); FABMS (m/z): 771 $(M+1)^+$.

2-Amino-6-(2-anilinoethyl)-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (16). A solution of aniline (1.5 mg, 17 μmol), camphorsulfonic acid (CSA, 2.0 mg, 8.6 μmol) and **7** (10 mg, 16 μmol) in dichloromethane was stirred at room temperature for 1 h, then the solvent was evaporated. The residue was chromatographed on a silica gel column (chloroform:methanol =99:1) to give **16** as a colorless oil (10 mg, 90%). IR (cm^{-1}, neat): 3400, 1740, 1600; ^{1}H-NMR ($CDCl_3$) δ: 8.10 (1H, s), 7.17-7.11 (2H, m), 6.69-6.62 (3H, m), 5.93 (1H, d, *J*=4.6 Hz), 4.97 (2H, brs), 4.55 (1H, t, *J*=4.3 Hz), 4.30 (1H, t, *J*=4.0 Hz), 4.11 (1H, dd, *J*=6.3, 3.6 Hz), 3.99 (1H, dd, *J*=11.5, 4.0 Hz), 3.78 (1H, dd, *J*=11.6, 3.0 Hz), 3.60 (2H, t, *J*=6.6 Hz), 3.32 (2H, t, 6.9 Hz), 0.96 (9H, s), 0.93 (9H, s), 0.80 (9H, s), 0.14 (3H, s), 0.13 (3H, s), 0.11(3H, s), 0.10 (3H, s), -0.03 (3H, s), -0.13 (3H, s); FABMS (m/z): 729 $(M+1)^+$.

2-Amino-6-(2-methoxyethyl)-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-purine (17). A solution of CSA (3.8 mg, 15 μmol) and **7** (9 mg, 15 μmol) in methanol (0.5 mL) was stirred at room temperature for 30 h, then the solvent was evaporated. The residue was chromatographed on a silica gel column (hexane:ethyl acetate=2:1 to 1:1) to give **17** as a colorless oil (4.4 mg, 44%). IR (cm^{-1}, neat): 3300, 3200, 1650, 1600; ^{1}H-NMR ($CDCl_3$) δ: 8.13 (1H, s), 5.92 (1H, d, *J*=5.0 Hz), 5.15 (2H, bs), 4.52 (1H, t, *J*=5.0 Hz), 4.27 (1H, t, *J*=4.3 Hz), 4.11 (1H, dd, *J*=6.1, 3.6 Hz), 4.00-3.91 (4H, m), 3.40-3.33 (2H, m), 3.35 (3H, s), 0.95 (9H, s), 0.93 (9H, s), 0.81 (9H, s), 0.13 (3H, s), 0.12 (3H, s), 0.10 (6H, s), -0.03 (3H, s), -0.21 (3H, s); FABMS (m/z): 668 $(M+1)^+$.

The adduct of 7 with 2',3',5'-tri-*O*-*tert*-butyldimethylsilylcytidine (18). A solution of 2',3',5'-tri-*O*-*tert*-butyldimethylsilylcytidine (47 mg, 80 μmol), CSA (10 mg, 40 μmol) and **7** (52 mg, 80 μmol) in dichloromethane was stirred at room temperature for 4 h, then the solvent was evaporated. The residue was chromatographed on a silica gel column (chloroform:methanol=99:1 to 95:5) to give **18** as a colorless syrup (16 mg, 17%) and the recovered **7** (21 mg, 41%). IR (cm^{-1}, neat): 3300, 3200, 1650, 1600; ^{1}H-NMR ($CDCl_3$) δ: 8.02 (1H, s), 7.45 (1H, d, *J*=7.6 Hz), 5.95 (1H, d, *J*=3.6 Hz), 5.90 (1H, d, *J*=4.6 Hz), 5.72 (1H, br), 5.03 (2H, brs), 4.58 (1H, t, *J*=4.6 Hz), 4.54-4.40 (2H, m), 4.30 (1H, t, *J*=4.3 Hz), 4.13-3.99 (5H, m), 3.93 (1H, dd, *J*=12.2, 3.0 Hz), 3.79-3.67 (2H, m), 3.40 (2H, t, *J*=7.6 Hz), 0.95 (9H, s), 0.94 (9H, s), 0.93 (9H, s), 0.91 (9H, s), 0.87 (9H, s), 0.82 (9H, s), 0.16 (3H, s), 0.15 (3H, s), 0.12 (6H, s), 0.10 (6H, s), 0.08 (3H, s), 0.07 (3H, s), 0.05 (3H, s), 0.04 (3H, s), -0.03 (3H, s), -0.17 (3H, s); FABMS (m/z): 1221 $(M+1)^+$; HRFABMS calcd for $C_{57}H_{113}N_8O_9Si_6$ 1221.7246, found 1221.7245.

The adduct of 7 with 2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine (19). A solution of 2',3',5'-tri-*O*-*tert*-butyldimethylsilylguanosine (80 mg, 130 μmol), CSA (32 mg, 130 μmol) and **7** (100 mg, 160 μmol) in dichloromethane was stirred at room temperature for 4 h, then the solvent was evaporated. The residue was chromatographed on a silica gel column (chloroform:methanol=99:1 to 95:5) to give **19** as a colorless syrup (54 mg, 27%) and the recovered **7** (10 mg, 10%). IR (cm^{-1}, neat): 3300, 3200, 1650, 1600; ^{1}H-NMR ($CDCl_3$) δ: 12.58 (1H, s), 9.15 (1H, s), 8.37 (1H, s), 5.93 (1H, d, *J*=5.0 Hz), 5.90 (1H, d, *J*=3.3 Hz), 5.10 (2H, bs), 4.98 (2H, bs), 4.61 (1H, m), 4.40 (1H, t, *J*=4.3 Hz), 4.24 (2H, t, *J*=4.3 Hz), 4.23-4.12 (2H, m), 4.08-3.91 (2H, m), 3.89-3.60 (6H, m), 3.43 (1H, d, *J*=14.5 Hz), 2.90 (1H, d, *J*=14.5 Hz), 2.64 (1H, brt), 2.32 (1H, brd), 2.05-1.89 (3H, m), 1.58-1.38 (2H, m), 1.10 (6H, s), 0.97 (9H, s), 0.95 (3H, s), 0.93

(9H, s), 0.92 (9H, s), 0.91 (9H, s), 0.87 (9H, s), 0.83 (9H, s), 0.16 (3H, s), 0.14 (3H, s), 0.13 (6H, s), 0.10 (6H, s), 0.09 (3H, s), 0.08 (3H, s), 0.01 (3H, s), -0.01 (3H, s), -0.05 (3H, s), -0.16 (3H, s); FABMS (m/z): 1261 $(M)^+$, 636 $(M-624)^+$.

General procedure for the reaction with *n*-butylamine in Scheme 3. The reaction was done using the vinyl compounds in 0.1 M and butylamine in 1 M concentrations in chloroform at room temperature, with monitoring the disappearance of the starting vinyl compounds by reversed-phase HPLC (Column: nakalai tesque COSMOSIL 5C18-MS, flow rate: 1 mL/min, eluent: CH_3CN:H_2O=4:1 including 0.05% CF_3COOH).

2-Amino-6-(2-butylaminoethyl)-9-(3,5-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-β-ribofuranosyl)purine. The crude residue was chromatographed on a silica gel column (chloroform:methanol=95:5 to 9:1) to give the adduct as a yellow oil in 46% yield. IR (cm^{-1}, neat): 3300, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.03 (1H, s), 6.32 (1H, t, *J*=6.6 Hz), 5.51 (2H, bs), 4.59-4.57 (1H, m), 3.83 (1H, dd, *J*=11.0, 4.0 Hz), 3.75 (1H, dd, *J*=11.0, 3.0 Hz), 3.52-3.43 (2H, m), 3.13 (2H, t, *J*=7.6 Hz), 2.98 (2H, t, *J*=7.6 Hz), 2.68-2.54 (1H, m), 2,45-2.29 (1H, m), 1.89-1.56 (4H, m), 0.96 (3H, t, *J*=7.3 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.11 (6H, s), 0.09 (3H, s), 0.08 (3H, s); FABMS (m/z): 579 $(M+1)^+$.

2-Amino-6-(2-butylaminopropyl)-9-(3,5-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl)purine. The crude residue was chromatographed on a silica gel column (chloroform:methanol=95:5 to 9:1) to give the adduct in 43% yield as a yellow oil together with the recovered **10** (53%). IR (cm^{-1}, neat): 3300, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.03 (1H, s), 6.32(1H, t, *J*=6.6 Hz), 5.63 (1H, bs), 5.61 (1H, bs), 4.59-4.57 (1H, m), 3.84 (1H, dd, *J*=11.0, 4.0 Hz), 3.65 (1H, dd, *J*=11.0, 2.6 Hz), 3.45-3.35 (2H, m), 3.19-3.01 (3H, m), 2.68-2.54 (1H, m), 2,45-2.29 (1H, m), 1.89-1.56 (4H, m), 1.52 (3H, d, *J*=6.6 Hz), 0.96 (3H, t, *J*=7.3 Hz), 0.92 (9H, s), 0.91 (9H, s), 0.11 (6H, s), 0.09 (3H, s), 0.08 (3H, s); FABMS (m/z): 593 $(M+1)^+$.

2-Isobutyrylamino-9-{2-deoxy-5-*O*-dimethoxytrityl-3-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl-phosphoramidyl)-β-D-ribofuranosyl}-6-(1-propenyl)purine (20). Under argon, a solution of **13** (496 mg, 0.7 mmol) and $n$$Bu_4NF$ (1M THF solution, 2.1 mL, 2.1 mmol) in THF (7.0 mL) was stirred at room temperature for 2 h, then the solvent was evaporated. The residue was chromatographed on a silica gel column (chloroform:methanol=100:0 to 98:2) to give the alcohol derivative (215 mg, 86%) as a colorless oil. A solution of the above alcohol (51 mg, 0.14 mmol) and dimethoxytrityl chloride (61 mg, 0.18 mmol) in pyridine (0.2 mL) was stirred for 3 h at room temperature, and diluted with chloroform, then washed successively with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), then evaporated to give the crude product, which was chromatographed on a silica gel column (chloroform:methanol=100:0 to 99:1) to the tritylated compound (73 mg, 92%) as a pale yellow. Under argon, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoamidite (0.03 mL, 0.12 mmol) was added into a solution of the above product (30 mg, 0.045 mmol) and diisopopylethylamine (0.04 mL, 0.24 mmol) in dichloromethane (0.4 mL) at 0 °C, and the mixture was stirred at the same temperature for 1 h. The mixture was diluted with dichloromethane (40 mL) and washed successively with saturated aqueous $NaHCO_3$ (10 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4) and evaporated to give the crude product, which was chromatographed on a silica gel column (hexane:ethyl acetate=2:1) to give the mixture of two diastereomers, (24 mg, 60%) as a colorless oil. IR (cm^{-1}, neat): 3300, 1650, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.11 (1H, s), 7.82 (1H, s), 7.50 (1H, dq, *J*=15.5, 6.9 Hz), 7.40-7.14 (9H, m), 6.92 (1H, dq, *J*=15.5, 1.7 Hz), 6.78 (2H, d, *J*=1.7 Hz), 6.75 (2H, d, *J*=1.7 Hz), 6.42 (1H, dd, *J*=7.3, 6.0 Hz), 4.81-4.72 (1H, m), 4.31-4.27 (1H, m), 3.77 (6H, s), 3.67 (2H, q, *J*=6.3 Hz), 3.74-3.56 (2H, m), 3.39 (1H, dd, *J*=10.2, 4.3 Hz), 3.37 (1H, dd, *J*=10.2, 4.3 Hz), 3.15-2.86 (2H, m), 2.65-2.58 (1H, m), 2.44 (2H, t. *J*=6.3 Hz), 2.05 (3H, dd, *J*=6.9, 1.7 Hz), 1.23 (6H, d, *J*=7.3 Hz), 1.19 (6H, d, *J*=6.9 Hz), 1.17 (6H, d, *J*=6.6 Hz); FABMS (m/z): 864 $(M^+ +1)$; HRFABMS calcd for $C_{47}H_{59}O_7N_7P$ 864.4214, found 864.4210.

2-Isobutyrylamino-9-{2-deoxy-5-*O*-dimethoxytrityl-3-*O*-(*N*,*N*-diisopropyl-β-cyanoethyl-phosphoramidyl)-β-D-ribofuranosyl}-6-(2-trimethylsilylvinyl)purine (21). The title compound was synthesized similarly as descried above from **14** (252 mg, 0.345 mmol) in 45% overall yield (20 mg) as a colorless oil. IR (cm^{-1}, neat): 3300, 1650, 1590; ^{1}H-NMR ($CDCl_3$) δ: 8.13 (1H, s), 7.95 (1H, s), 7.82 (1H, d, *J*=19.1 Hz), 7.43 (1H, d, *J*=18.9 Hz), 7.40-7.14 (9H, m), 6.78 (2H, d, *J*=1.7 Hz), 6.75 (2H, d, *J*=1.7 Hz), 6.45 (1H, dd, *J*=7.3, 6.0 Hz), 4.74-4.56 (1H, m), 4.21-4.25 (1H, m), 3.76 (6H, s), 3.38 (2H, q. *J*=6.6 Hz), 3.74-3.56 (4H, m), 2.90-2.78 (2H, m), 2.77-2.66 (1H, m), 2.65 (2H, t, *J*=6.3 Hz), 1.23 (6H, d, *J*=7.3 Hz), 1.19 (6H, d, *J*=6.9 Hz), 1.17 (6H, d, *J*=6.6 Hz), 0.22 (9H, s); FABMS (m/z): 922 $(M^+ +1)$; HRFABMS calcd for $C_{49}H_{64}O_7N_7PSi$ 922.4452, found 922.4453.

UV Measurement and Determination of p*Ka*. UV spectra were measured using a 0.03 mM solution of the compound in 0.1 M aqueous KCl solution at 30 °C containing 0.1% MeOH. The p*Ka* values were calculated based on the curves which were obtained by plotting absorbances at 230, 350 nm against pH, and summarized below. The wave length of the maximum absorbance (λ_{max}) and the extinction coefficient (ε_{max}) were obtained at pH indicated in parenthesis.

Compound	p*Ka*	λ_{max}	ε_{max}
deprotected-**9**	3.7	333 (pH 6.47)	6343
		229	25179
deprotected-**10**	4.0	328 (pH 6.34)	7362
		227	21563
deprotected-**11**	3.9	333 (pH 6.50)	8055
		226	25420

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